# A bottom-up approach to identifying sources of instability in a mutualistic association between *Methanococcus maripaludis* and *Desulfovibrio vulgaris*


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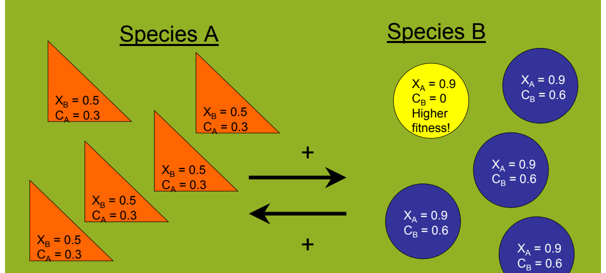
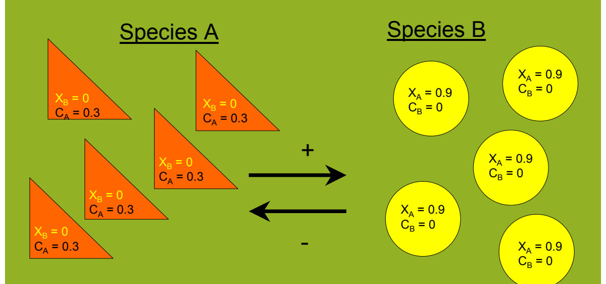
## I. Factors affecting the stability of mutualistic interactions

Interspecies interactions are abundant in the microbial world. In some of these interactions, species provide a net benefit to one another at some cost to themselves. However, the stability of such mutualistic interactions may be under constant threat of invasion by mutants that can receive the benefit from a species without reciprocating. Consider the following scenario:

Two species, A and B, exchange metabolites, such that species A increases the fitness of species B by $X_A$, and species B increases the fitness of species A by $X_B$. Providing these benefits comes at a cost, C, to each species as depicted below.




Since the yellow variant has higher fitness, after several generations in the same environment, the relationship between species A and species B will be something like this:




Key
$X_A$ = Benefit provided by species A
$X_B$ = Benefit provided by species B
$C_A$ = cost to A of providing benefit to B
$C_B$ = cost to B of providing benefit to A
Total fitness = fitness + X - C
Yellow = genetic variant in population that does not provide benefit to the other species

Thus, mutualistic interactions are susceptible to conflicts between the species in the properties of the interaction that would be most beneficial to them. This can lead to parasitic interactions or invasion of the mutualism by third parties. Other examples of such conflicts include:

1. The rate of exchange of goods. In the case of exchange of resources, it is always ideal for a species to provide less resource to its partner if it can get the same benefit, just as it is in your best interest to pay less money for the same number of pipet tips or other products.
2. The quality of the goods being exchanged. For example, in the case of molecules being exchanged between species, it may be preferable for species B to receive the molecule with a particular functional group removed from it, but it may be more costly for species A to remove it.

## II. The mutualism

*Desulfovibrio vulgaris Hildenborough* (DvH) and *Methanococcus maripaludis* (Mm) were incubated together at 30ºC in the absence of sulfate and hydrogen in B3n media which includes a bicarbonate buffer and 25mM lactate. In this environment, the energy-yielding reactions are as follows:

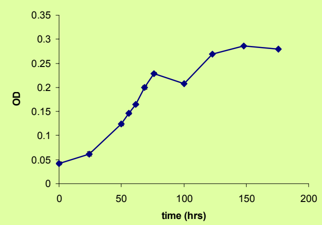
1. 2 lactate + $4H_2O \rightarrow$ 2 acetate + $2H^+$ + $2HCO_3^-$ + $4H_2$ (DvH)
2. $4H_2$ + $H^+$ + $HCO_3^-$ $\rightarrow$ $CH_4$ + $3H_2O$ (Mm)

Total: 2 lactate + $H_2O \rightarrow$ 2 acetate + $CH_4$ + $H^+$ + $HCO_3^-$

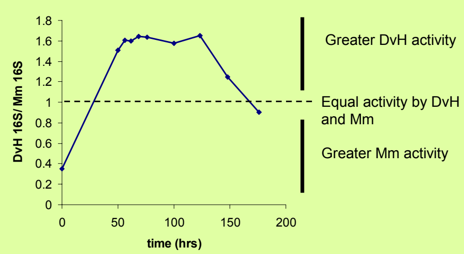
$\Delta G = -144$ kJ

The first reaction is not favorable in the absence of Mm and the second is impossible in the absence of DvH since no hydrogen is provided in the culture environment. When both organisms are incubated together, consumption of hydrogen by Mm makes the first reaction thermodynamically feasible, allowing both organisms to obtain energy.

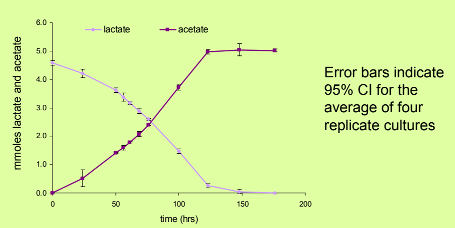
Growth dynamics of co-culture



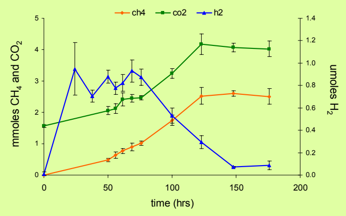

Relative activity of DvH and Mm over time




Secretion and uptake of metabolites in coculture

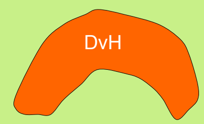



Error bars indicate 95% CI for the average of four replicate cultures

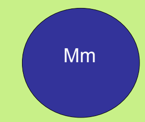



## III. Hypothetical conflicts between DvH and Mm

We present below two hypothetical conflicts that may affect the stability and evolution of the syntrophic interaction between DvH and Mm. The conflicts in these situations may arise from a difference between DvH and Mm in the cost and benefit of each option. These hypotheses have not been tested, but are presented to illustrate the types of conflicts that could occur in a syntrophic association.




1. Which electron carrier: Formate, Hydrogen, Other, or some combination? It could be more costly for DvH to use formate than hydrogen, but electrons carried as formate may provide a greater benefit to Mm than hydrogen.
2. Which electron transfer rate? Perhaps it is more energetically expensive for DvH to release hydrogen into the environment at very high rates, but such high flux rates could significantly improve growth rate of Mm relative to a lower flux rate environment.




Natural selection would favor any Mm genotype that could force associated DvH to release electrons more quickly or to use a preferred electron carrier that is costly to DvH. Once such mutants predominate in the population, natural selection would favor any DvH that were capable of resisting such manipulation, leading to an evolutionary tug-of-war. If the most beneficial form of electron transfer for Mm was also the least costly for DvH, then there would be no such tug-of-war.

## IV. Exploration of the potential of flux balance modeling to predict sources of conflict.

Normally biologists would investigate potential costs, benefits, and sources of conflict in a mutualism by collecting data on variation in behavior and its influence on population dynamics, and then following up with experimentation and comparison to theoretical predictions. But perhaps there is another way to research such possibilities:

**Is it possible to predict sources of conflict from the organism's gene content and the stoichiometry of metabolic reactions?** This may be accomplished by utilization of a flux-balance model. A flux-balance model consists of a network of metabolic reactions of known stoichiometry and the flux rate of metabolites through metabolic pathways. The metabolic reactions within the network are chosen based on what is known from physiological studies and the genome sequence. Flux rates are calculated from experimentally determined constraints and linear optimization of an objective function, such as biomass. A flux-balance model of the coculture has already been developed.

**How might a flux-balance model be used to predict sources of conflict in a syntrophic mutualism?** Optimization of the model to the biomass of one or the other species, should result in calculated flux rates that maximize the growth and fitness of that organism. If the flux rates of electron carriers are the same regardless of the optimization function, then it is unlikely that there would be a conflict over electron transfer modes. If they are different, then this may represent a potential source of conflict that could be investigated further by experimentation.

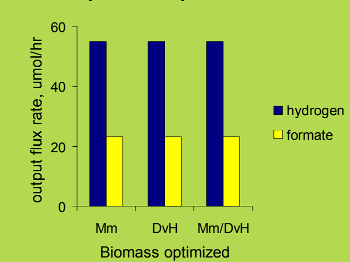
Initial application of flux-balance model to exploring potential for electron carrier conflicts:
Flux rates from exponential and stationary phase measurements of lactate, acetate, and methane in panel II were used as constraints. The model was optimized to the following objective functions:

**Objective functions**
1. 0 DvH +1.0 Mm biomass
2. 1.0 DvH + 0 Mm biomass
3. 0.5 DvH + 0.5 Mm biomass

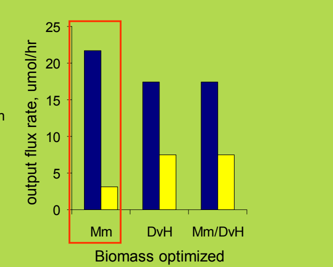
**Model constraints, umol/hr**

| | Exp | Stat |
|---|---|---|
| Lactate | 39.5 | 8.26 |
| Acetate | 37.5 | 2.65 |
| Methane | 18.6 | 3.4 |

**Flux predictions in exponential phase**




**Flux predictions in stationary phase**




Conclusions:
These preliminary results suggest that the hydrogen and formate flux rates which optimize growth of Mm during stationary phase are not the same as those that optimize growth of DvH. Optimization of Mm biomass during stationary phase requires a greater hydrogen flux rate and a lower formate flux rate than optimization of DvH biomass.

**These model results might indicate a conflict between DvH and Mm that could affect their evolution.** However, this possibility must be explored further with additional simulations and verification of the accuracy of the model.

Additional potential sources of conflict could be explored by comparing the flux rates of additional metabolites under these optimization criteria, or by exploring more diverse environmental conditions, such as a $CO_2$ limiting environment.